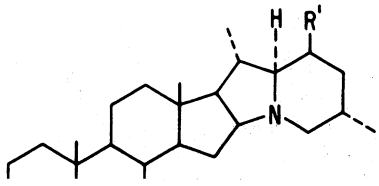
THE METABOLISM OF SOLANIDINE BY MICROSOMAL FRACTIONS FROM *SOLANUM CHACOENSE*

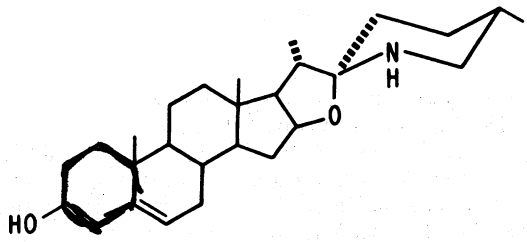
Abstract—Microsomal fractions prepared from leaves of selected clones of *Solanum chacoense* after addition of NADPH converted solanidine to a 23β-hydroxysolanidine glycoside and an unknown compound apparently an isomeric hydroxysolanidine.

INTRODUCTION

Tubers of *Solanum chacoense* Bitt. contain the steroidal alkaloid glycosides solanine (**1a**) and chaconine (**1b**), compounds also found in the cultivated species, *S. tuberosum*. The aerial portions of some *S. chacoense* clones (this species is a hybrid) contain the analogous 23β-acetoxy compounds, leptine I (**1c**) and the 23β-hydsroxy compounds leptinine I (**1d**, chacotriose) and leptinine II (**1d**, solatriose) [1, 2]. The leptines and to a lesser degree the leptinines are important factors in the resistance of *S. chacoense* to the Colorado potato beetle [1–3]. On the basis of precursor feeding experiments it was shown that solanidine (**1e**) and the closely related spirosolane tomatid-5-en-3β-ol (**2**) are biosynthetically derived from cholesterol [cf. 2, 4].

Substitution at C-23 can occur on many of the proposed intermediates. 23β-hydroxysolanidine (leptinidine, **1f**) has been chemically synthesized from tomatid-5-en-3β-ol (**2**) [5]. The purpose of this study was to determine if solanidine (**1e**) can be enzymatically converted to 23β-hydroxysolanidine (**1f**) by *S. chacoense* clones that synthesize the leptine glycoalkaloids.





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	R	R′
1a	Solatriose*	H
1b	Chacotriose*	H
1c	Chacotriose*	O_2CMe
1d	Solatriose or chacotriose*	OH
1e	H	H
1f	H	OH

*Solatriose = *O*-α-L-rhamnopyranosyl-(1 → 2 gal)-*O*-β-D-glucopyranosyl(1 → 3 gal)-β-D-galactopyranose

*Chacotriose = *O*-α-L-rhamnopyranosyl-(1 → 2 glu)-*O*-α-L-rhamnopyranosyl (1 → 4 glu)-β-D-glucopyranose

RESULTS AND DISCUSSION

[^{14}C]-Solanidine was incubated with microsomal fractions derived from leaf tissue of *S. chacoense* clones that produce the leptine glycoalkaloids. For reasons to be discussed later, two other incubation media, whole leaf and cell free homogenates of leaves, were not satisfactory for biosynthetic studies. After 2 hr at 25° (with NADPH added to the media) *ca* 5–10% of the recovered radioactivity was found in compounds other than solanidine (**e**) (Fig. 1). The compounds were isolated by preparative TLC. Radioactive zones were removed from the TLC plate and eluted with $CHCl_3$–MeOH (1:1). The eluants

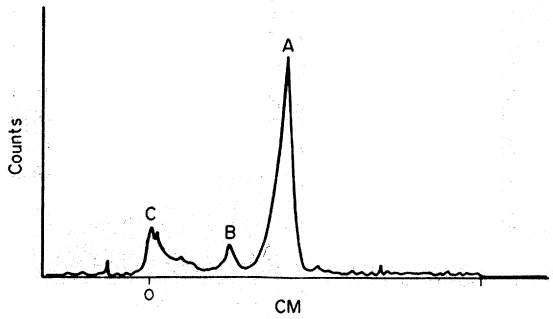

Fig. 1. TLC radiochromatogram of $CHCl_3$–MeOH extract of [^{14}C]-solanidine–*S. chacoense* microsomes incubation. Mobile phase, cyclohexane–EtOAc (1:1). A: Solanidine (1e), B: unknown hydroxysolanidine, C: glycosylated alkaloids.

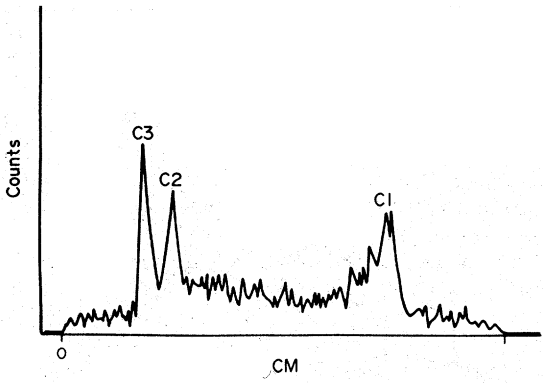

Fig. 2. TLC radiochromatogram of $CHCl_3$ extract of C (Fig. 1) after hydrolysis with 5% HCl–MeOH. Mobile phase, cyclohexane-ETOAc (1:1). C1: solanidine (1e), C2: 23β-hydroxysolanidine (1f), C3: apparently identical with compound C (cf. Fig. 1).

were analysed by direct probe-mass spectrometry (DPMS). Compound B (Fig. 1) had an apparent M_r of 413, consistent with that of a hydroxylated solanidine, however the mass spectrum differed from that of authentic 23β-hydroxysolanidine particularly with respect to ions derived from fragmentation of the E and F rings (i.e. 23β-hydroxysolanidine spectrum contains ions *m/z* 166 and 220 which are apparently the hydroxy analogues of *m/z* 150 and 204 in the spectrum of solanidine [4, 6]; none of these ions are present in the spectrum of the unknown compound). Compound C gave no significant ion current when analysed by DPMS. The radiogram of C after hydrolysis with 5% HCl in MeOH is shown in Fig. 2. The radioactive zones were isolated by preparative TLC and analysed by DPMS. Compound C1 was identified as solanidine (**1e**) and C2 as 23β-hydroxysolanidine (**1f**) Compound C3 was apparently unhydrolysed C since it had the same TLC and DPMS characteristics as C. The behaviour of compound(s) C is consistent with that of a glycoside. However one would not expect the microsomal pellet to contain the necessary precursors for glycosylation. We have considered the possibility that leptine glycoalkaloids may contaminate the microsomal fraction

Table 1. Summary of ^{14}C incubation experiments

Substrate	Enzyme source	Co-factor	Products
Solanine and chaconine	Microsomes	NADPH	—
Solanidine	Microsomes	NADPH	After hydrolysis 23β-hydroxysolanidine and unknown hydroxy-solanidine
Solanidine	Boiled microsomes	NADPH	—
—	Microsomes	NADPH	—*
Solanidine	Microsomes	—	—

*Control for DP-MS analysis, i.e. no 23β-hydroxysolanidine was detected by DP-MS in extracts of the pellet either before or after hydrolysis with 5% HCl–MeOH.

(a problem that precludes the use of whole leaf or cell free extracts of leaves as an enzyme source). The microsomal pellet has therefore been washed twice with water to remove any glycoalkaloid contaminant (Table 1). Evidence has been presented for the biosynthetic conversion of solanidine to the *Veratrum* alkaloids jervine and veratramine via a pathway that includes glycosylation and 12-hydroxylation of solanidine [7]. The structures of the conjugates we have isolated remain to be characterized although it is evident that the normal metabolites are found only in this form since the unconjugated metabolite (B, Fig. 1) is not native to the plant. It is interesting that an unknown metabolite with M_r 413 has also been isolated in the *Veratrum* studies previously cited.

The enzymatic hydroxylation does not occur with glycoalkaloid or in the absence of NADPH, suggestive of cytochrome P-450 involvement (Table 1).

EXPERIMENTAL

Mass spectra : direct probe . Radioactive zones on TLC plates were detected using a Berthold LB 2832 automatic TLC linear analyser. All TLC's were performed on silica gel G plates (Analtech). *S. chacoense* plants were grown in the laboratory under mixed indirect sunlight and fluorescent lighting.

Microsomal fraction. Leaves (3.7 g) from 2 month old plants were ground in 12 ml of a soln containing 0.1 M HEPES buffer pH 7.5, 0.3 M sucrose, 8 mM dithioerythritol and 8 mM β-mercaptoethanol. The suspension was centrifuged (13 000 *g*) for 15 min, the supernatant removed and centrifuged at 100 000 *g* for 50 min. The pellet was resuspended in 10 ml of the above buffer soln (except the concn of dithioerythritol and β-mercaptoethanol was 5 mM) and recentrifuged at 100 000 *g* for 50 min; after repeating this process again the pellet was resuspended for use.

Preparation of [^{14}*C*]*-solanine and* [^{14}*C*]*-chaconine.* Uniformly labelled [^{14}C]-sodium acetate soln (200 μl, 4×10^7cpm) was applied to each of 10 freshly prepared potato (Katahdin variety) tuber slices. The slices were stored individually in Petri dishes and after 4 days at room temp. the slices were quartered and then ground in a Waring blender with 250 ml of 1% HOAc–MeOH. After filtration on a medium scintered funnel and centrifugation (10 000 *g*) of the filtrate, the soln was neutralized with con NH_4OH. After heating at 50° for 15 min the soln was stored at 10 for 16 hr. The suspension was then centrifuged (10 000 *g*), the ppt suspended in 1% NH_4OH, recentrifuged (10 000 *g*), filtered and

ashed with acetone and $CHCl_3$. The solid (29 mg) was dissolved n 1% HOAc–MeOH (4 ml) and separated by TLC (upper layer from $CHCl_3$–MeOH (1:1) saturated with 0.5 pts 1% NH_4OH, nobile phase). The total activity was 800 cpm/μg solanine + chaconine.

The MeOH soln was concd to dryness and the residue taken up in 5% HCl in MeOH, then heated for 2 h (70°). After neutralization with M NaOH, the soln was concd to 75% of the original vol then extracted 2x with C_6H_6 (equal vol.). After concn the residue was taken up in 95% EtOH (5 ml). TLC (cyclohexane–EtOAc, 1:1) indicated > 95% of the activity in solanidine (600 cpm/μg) and the rest of the activity co-chromatographing with solanthrene.

Incubation and metabolite isolation: A suspension of 900 μl of [^{14}C]-solanidine (1×10^7 cpm) in 1 ml of H_2O containing 2.9 $\times 10^{-2}$ mg of octylglucoside (and NADPH where noted) was added to 1 ml of microsomal suspension and shaken for 2 hr at 25° in an open test tube. The soln was then diluted with twice the vol. of $CHCl_3$–MeOH (1:1) and vortexed for 1 min. The layers were separated and the aq. layer was concd to dryness under a stream of N_2, taken up in $CHCl_3$–MeOH (1:1) and combined with the $CHCl_3$ layer. After concn under N_2 the residue was taken up in 1 ml of $CHCl_3$–MeOH (1:1). The extract was subjected to prep TLC using cychohexane–EtOAc (1:1) as the mobile phase. Radioactive zones (Fig. 1) were eluted with $CHCl_3$–MeOH (1:1), concd and analysed by DPMS. The radioactive zone C of least mobility was subjected to hydrolysis with 5% HCl in MeOH (2 hr at 70°), re-chromatographed and the zones of activity (Fig. 2) were eluted.

Metabolite characterization. The metabolites were characterized by DPMS and chromatographic behaviour in (a) cyclohexane–EtOAc (1:1), R_f solanidine 0.85, 23β-hydroxysolanidine 0.31 and (b) MeOH-$CHCl_3$ (1:19), R_f solanidine 0.54, 23β-hydroxysolanidine 0.40. It should be noted that the TLC mobility of solanidine can be variable in $CHCl_3$–MeOH extracts of leaf tissue or cell free extracts derived from leaves. Initial incubation experiments using leaf tissue or cell free extracts resulted in misleading results. The R_f of solanidine added to $CHCl_3$–MeOH extracts was invariably different from the R_f of solanidine extracted from solns of solanidine incubated with microsomes. Only by re-chromatography and DPMS analysis was this anomaly made evident. Solvents (a) and (b) were used for prep. chromatography and radioactive zones were eluted with $CHCl_3$–MeOH (1:1). MS identification was made by comparison with spectra of authentic compounds (solanidine: *m/z* 397, 382, 379, 204, 150; 23β-hydroxysolanidine *m/z* 413, 398, 220, 166). The MS of the unidentified metabolite had the following major ions: *m/z* 413, 385, 138, 114, 113.

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